APPENDIX N2
Quality-control samples for RT-PCR analysis

SAMPLE TYPE	DESCRIPTION	PURPOSE
Positive-control seed (done in the field)	Poliovirus vaccine injected to a second environmental sample during filtration	Recovery efficiency Inhibitor removal efficiency Cross-contamination of samples during processing and analysis
Equipment blank	Sterile water subjected to all phases of sample collection, handling, processing and analysis	Adequacy of equipment cleaning and disinfection and contamination potential during sample collection and processing
Negative-process control	40 mL of sterile water analyzed with each batch of five samples beginning at inhibitor removal	Cross-contamination of samples during inhibitor removal, RT-PCR, and hybridization
Seeded negative-process control	Virus mixes added to a duplicate negative process control with each batch of five samples before RT-PCR	Verify proper completion of RT-PCR reaction
Matrix spikes	Virus mixes added to duplicate environmental samples before RT-PCR	Indicates whether inhibitors in environmental samples were present
PCR positive controls	RT-PCR products from positive control virus mixes added to sterile water (also used as hybridization positive control)	Verify the proper completion of the RT-PCR and hybridization reaction
PCR negative controls	Sterile water added at the RT-PCR step	Contamination during RT-PCR
Hybridization negative controls	Sterile water added to hybridization wells	Cross-contamination of sample during hybridization
Cell-culture assays	A volume of eluate obtained after elution and concentration and analyzed by the cell-culture method	Recovery efficiency

QUALITY-CONTROL SAMPLE CHECK LIST FOR RT-PCR Last analytical date _____

SAMPLE NAME						
Sample results		Ent Reo Rot HAV Nor	Ent Reo Rot HAV Nor	Ent Reo Rot HAV Nor	Ent Reo Rot HAV Nor	Ent Reo Rot HAV Nor
Negative-process control	Ent Reo Rot HAV Nor					
Seeded negative-process control	Ent Reo Rot HAV Nor					
Matrix spikes		Ent Reo Rot HAV Nor	Ent Reo Rot HAV Nor	Ent Reo Rot HAV Nor	Ent Reo Rot HAV Nor	Ent Reo Rot HAV Nor
Hybridization positive controls	Ent Reo Rot HAV Nor					
Hybridization negative controls	Ent Reo Rot HAV Nor					
Cell culture						

Negative-process control--40 mL of sterile water analyzed with other samples beginning at inhibitor removal.
Seeded-negative process control--negative-process control seeded with virus stocks before RT-PCR.
Matrix spikes-virus mixes added to duplicate environmental samples before RT-PCR.
Hybridization positive controls--RT-PCR products from virus mixes added to sterile water.
Hybridization negative controls--Sterile water added to hybridization wells.
ADD THE FOLLOWING NUMBERS WHEN APPROPRIATE:

1. Inconclusive because matrix spike was not detected.
2. Inconclusive because negative process control was positive and signal was not greater than negative process control.
3. Inconclusive because PCR reaction was incomplete.